

Long-Term Outcomes after Phthalate Exposure

Food Intake, Weight Gain, Fat Storage, and Fertility in Mice

Exposure to endocrine-disrupting chemicals (EDCs), particularly *in utero*, is suspected to contribute to obesity, diabetes, hypertension, and reproductive abnormalities. Di(2-ethylhexyl) phthalate (DEHP), a plasticizer found in cosmetics, fragrances, food packaging, and polyvinyl chloride, is one such EDC. Human studies have found associations between urinary metabolites of DEHP and other phthalates and increased body mass in humans, and maternal exposure to DEHP has been associated with impaired gonadal development and fertility in baby boys. However, much less is known about potential effects of DEHP on female health. In a two-part investigation, researchers documented weight and fertility changes in female mice exposed to DEHP, and then documented how exposure *in utero* and during lactation affected their offspring [EHP 120(8):1123–1129; Schmidt et al.].

In the first study, adult female mice were given diets formulated to deliver one of three levels of DEHP (0.05, 5, or 500 mg/kg body weight) for 8 weeks. The lowest level was comparable to the tolerable daily intake for humans issued by the World Health Organization in 2003. Although outwardly healthy, dams fed all three levels of DEHP had significantly increased food intake, body weight, and visceral fat compared with controls. All treatment groups also showed increased gene expression of the hormone leptin (consistent with the animals' increased visceral fat) and decreased expression of adiponectin (which may suggest

potential effects on insulin sensitivity).

As in previous studies, investigators found that at the highest level of DEHP exposure, expression of peroxisome proliferator-activated receptors alpha and gamma (PPAR α and PPAR γ)—which are mediators of adipogenesis and fat metabolism—was increased in the liver. However, unlike previous studies, they found a decrease in PPAR α expression in visceral fat at the highest dose. The authors note that a pair-feeding design in which exposed and control animals receive the same amount of calories should be used to determine whether observed effects were a consequence of increased food intake or of metabolic effects of DEHP independent of caloric intake.

In the second study, the investigators found that DEHP-exposed pups of both sexes had higher body weight at weaning than nonexposed pups. Higher body weight persisted 9 weeks after exposure ceased, and fat storage was significantly higher in female adult pups in a dose-dependent manner. These findings were surprising because DEHP is rapidly metabolized and excreted, yet the results suggest a lingering effect of *in utero* exposure to DEHP on body weight and fat tissue formation.

At the highest DEHP exposure, a dose unlikely to be found in the environment, all dams experienced 100% spontaneous abortion. Surviving, lesser-exposed offspring were placed on a standard diet at weaning, and female offspring were mated to unexposed males. Although the total number of embryos was not reduced in pregnant females exposed to DEHP *in utero*, the investigators did find that 28% of the dams' blastocysts were not viable in the low-dose group and 29% were not viable in the middle-dose group, compared with just 8% in controls. However, the difference was not statistically significant.

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Filling a Gap in Developmental Toxicity Testing

Neural Crest Cells Offer Faster, Cheaper, Animal-Free Testing

The neuronal development of fetuses and infants is exquisitely sensitive to disruption by various environmental factors. Yet few chemicals in widespread use have been thoroughly tested for developmental neurotoxicity. Most such testing relies on animal studies, a laborious and costly process that is not always a good predictor of human health outcomes. A team of researchers now describes a faster, cheaper, and more humane approach to developmental neurotoxicity testing using human neural crest (NC) cells *in vitro* [EHP 120(8):1116–1122; Zimmer et al.].

NC cells separate from the neural tube and migrate during embryonic development, giving rise to a wide variety of cell types that form the peripheral nervous system, bone and cartilage in the head, and other

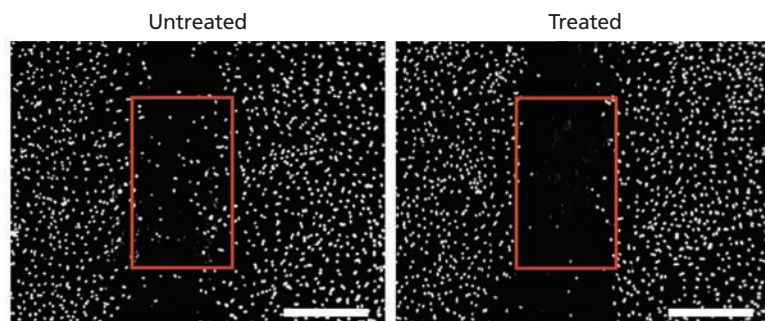
tissues. Certain drugs and environmental chemicals interfere with this migration, causing serious developmental defects.

The researchers based their new test, called the MINC (“migration of NC”) assay, on the previously established scratch assay. In this test, a gap is scratched into a monolayer of cells, and a chemical is added to measure its effect on the cells' attempts to migrate across the gap. The researchers showed that the MINC assay detected impairment of NC cell migration by the neurotoxicants methylmercury and lead-acetate with very high sensitivity. More important, the MINC assay—but not an assay using other neural precursor cells—detected the antiepileptic drug valproic acid in the low micromolar range. Valproic acid is a human reproductive toxicant known to interfere with NC cell migration in several species.

The researchers also substituted several other types of migratory cells for NC cells in the test, but none were as sensitive to methylmercury or lead-acetate. The specificity of the test to neurotoxicants was indicated by its detection of methylmercury, lead-acetate, valproic acid, and the fungicides triadimefon and triadimenol, whereas aspirin, acetaminophen, and mannitol (a sugar alcohol used in foods and medical applications) showed no effect on NC cells—all consistent with expectations. Moreover, three forms of mercury were ranked according to their potency as disruptors of NC cell migration, suggesting that the assay may be useful in predicting the toxicity potency of a broad range of compounds.

The researchers showed that NC cells can be produced from human embryonic stem cells in large quantities, frozen, and thawed for use in the MINC assay—features that would make them easy to transport and use in laboratories around the world. They envision the MINC assay as part of a suite of cell-based tests that together could be used to quickly assess numerous potential toxicologic effects of chemical compounds.

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Representative images show normal (untreated) and blocked (treated) cell migration on the MINC assay.